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(54) Title: VACCINE COMPOSITIONS COMPRISING THE *HELICOBACTER PYLORI* FlgE POLYPEPTIDE

(57) Abstract

The present invention relates to polypeptides and vaccine compositions for inducing a protective immune response to *Helicobacter pylori* infection. The invention furthermore relates to the use of *Helicobacter pylori* polypeptides in the manufacture of compositions for the treatment or prophylaxis of *Helicobacter pylori* infection.

VACCINE COMPOSITIONS COMPRISING THE *HELICOBACTER PYLORI* FlgE POLYPEPTIDE

TECHNICAL FIELD

The present invention relates to polypeptides and vaccine compositions for inducing a protective immune response to *Helicobacter pylori* infection. The invention furthermore relates to the use of *Helicobacter pylori* polypeptides in the manufacture of compositions for the treatment or prophylaxis of *Helicobacter pylori* infection.

BACKGROUND ART

Helicobacter pylori

The gram-negative bacterium *Helicobacter pylori (H. pylori)* is an important human pathogen, involved in several gastroduodenal diseases. Colonization of gastric epithelium by the bacterium leads to active inflammation and progressive chronic gastritis, with a greatly enhanced risk of progression to peptic ulcer disease. A lifelong inflammation of the gastric mucosa is very closely correlated with a significantly enhanced risk for gastric cancer.

In order to colonize the gastric mucosa, *H. pylori* uses a number of virulence factors. Such virulence factors comprise several adhesins, with which the bacterium associates with the mucus and/or binds to epithelial cells; urease which helps to neutralize the acid environment; and proteolytic enzymes which makes the mucus more fluid. In addition *H. pylori* is highly motile, swimming in the mucus and down into the crypts. Motility has been shown to be an essential virulence factor, since non motile *H. pylori* has failed to infect the mucosa in experimental models Eaton et al. (Infection & Immunity 64(7), 2445-2448, 1996).

There are many possible reasons for this, the most obvious being an inability to swim down and attach to mucosal cells and the inability to avoid noxious agents in the stomach.

Despite a strong apparent host immune response to *H. pylori*, with production of both local (mucosal) as well as systemic antibodies, the pathogen persists in the gastric mucosa, normally for the life of the host. The reason for this is probably that the spontaneously induced immune-responses are inadequate or directed towards the wrong epitopes of the antigens. Alternatively the immune response could be of the wrong kind, since the immune system might treat *H. pylori* as a commensal (as indicated from the life-time host/bacteria relationship).

In order to understand the pathogenesis and immunology of *H. pylori* infections, it is of great importance to define the antigenic structure of this bacterium. In particular, there is a need for characterization of surface-exposed, surface associated as well as secreted proteins which, in many bacterial pathogens, have been shown to constitute the main virulence factors, and which can be useful for the diagnosis of *H. pylori* and in the manufacture of vaccine compositions. If such proteins in addition to being surface associated also are essential for survival and/or colonization their usefulness as a target for vaccine mediated immunotherapy targets increase.

Whenever stressed or threatened, the *H. pylori* cell transforms from a bacillary to a coccoid form. In the coccoid form, the *H. pylori* cell is much less sensitive to antibiotics and other anti-bacterial agents. Circumstantial evidence indicate the *H. pylori* might be transmitted between individuals in this form, possibly via water or direct contact (oral-oral; feacal-oral). An efficient vaccine composition should therefore elicit an immune response towards both the coccoid and the bacillary form of *H. pylori*. Since systemic immunity probably only plays a limited role in

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protection against mucosal infections, it is also important that the vaccine composition will enhance protective immune mechanisms locally in the stomach.

Flagellar Hook protein

Flagellar hooks from *H. pylori* has been shown to be composed of FlgE subunits of 78 kDa (O'Toole et al. Molecular Microbiology, 14(4), 691-703, 1994). The role of the flagellar hook is to connect the flagella with the submembraneous flagellar motor. The part of the hook extruding outside the membrane is short, approximately 60 nanometers (compared to approximately 10 micrometers for the flagella). Like the fagellum of *H. pylori* the hook is probably covered with a sheet (Geis et al. (1993) J. Med. Microbiol. 38(5), 371-377).

The amino acid sequence of the FlgE polypeptide has significant resemblance with that of other known hook proteins, including limited homology to other *Helicobacter* species like *mustelae* (O'Toole et al., *supra*). Polyclonal antibodies raised against the FlgE polypeptide showed cross-reactivity against flagellar proteins A and B, possibly indicating the existence of shared epitopes. Production of FlgE knockout *H. pylori*, resulted in an aflagellar, non-motile bacteria, where FlgE polypeptide still was produced but could only be recovered in the cytoplasm.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1:
Effect of therapeutic immunization of *H. pylori* infected mice (n=9-10/group) with FlgE polypeptide. Results are given as mean±SEM of number of *H. pylori* associated with antrum (=**A**), corpus (=**B**) or totally (A+C) (=**C**). Abbreviations: CFU, colony forming units (number of bacteria); unshaded bars=DOC + CT, Phosphate buffered saline with 0.5% deoxycholate given together with cholera

toxin 10 µg/mouse; shaded bars=FlgE + CT, mice given 100 µg FlgE and 10 µg cholera toxin. The decrease in cfu was significant in the antrum and as calculated for the whole stomach.

** $p<0.01$; * $p<0.05$ (Wilcoxon-Mann-Whittney sign rank test).

Fig. 2:

Serum IgG from mice measured by ELISA technique: response to infection and to immunisation with FlgE. The values are expressed as mean titers ± SEM. n=9-10/group. ELISA coated with *H. pylori* strain 244: As a sign of infection *H. pylori* specific antibodies can be found in serum in animals treated with DOC + CT (=A. Control/244). Following immunization with FlgE + cholera toxin (=B. FlgE/244) this reactivity increased 4 fold (** $p<0.01$; Wilcoxon-Mann-Whittney sign rank test). C=FlgE specific. Specific FlgE IgG increased in animals given FlgE + CT, but could not be detected in control animals.

DISCLOSURE OF THE INVENTION

The purpose of this invention is to provide an antigenic *H. pylori* polypeptide which can be useful for eliciting a protective immune response against, and for diagnosis of, *H. pylori* infection. This purpose has been achieved by the recombinant cloning of an *H. pylori* gene which encodes a well conserved essential polypeptide. The nucleic acid sequence of this gene is similar to the sequence of the *flgE* gene as published by O'Toole et al., Molecular Microbiology, 14(4), 691-703, 1994. Being an essential protein for motility, the *flgE* gene is expressed by all *H. pylori* strains.

It has surprisingly been found that the *H. pylori* FlgE polypeptide, in spite of the facts that only a small part of the hook protein is existing outside bacteria and that it is probably covered by a sheet, can serve as a therapeutic antigen in an *H. pylori*

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to identify modified forms of the FlgE polypeptide retaining functionally equivalent antigenicity, by use of known methods, such as epitope mapping with *in vivo* induced antibodies.

In a preferred form of the invention, the *Helicobacter pylori* FlgE polypeptide, for use in inducing a protective immune response to *Helicobacter pylori* infection, has substantially the amino acid sequence set forth as SEQ ID NO: 2 in the Sequence Listing, or is a modified form thereof retaining functionally equivalent antigenicity.

It is thus to be understood that the definition of the *Helicobacter pylori* FlgE polypeptide is not to be limited strictly to a polypeptide with an amino acid sequence identical with SEQ ID NO: 2 in the Sequence Listing. Rather the invention encompasses polypeptides carrying modifications like substitutions, small deletions, insertions or inversions, which polypeptides nevertheless have substantially the biological activities of the *Helicobacter pylori* FlgE polypeptide and is retaining functionally equivalent antigenicity. Included in the definition of the *Helicobacter pylori* FlgE polypeptide are consequently polypeptides, the amino acid sequence of which is at least 90% homologous, preferably at least 95% homologous, with the amino acid sequence set forth as SEQ ID NO: 2 in the Sequence Listing.

In another aspect, the invention provides a vaccine composition for inducing a protective immune response to *Helicobacter pylori* infection, comprising an immunogenically effective amount of a *Helicobacter pylori* FlgE polypeptide as defined above, optionally together with a pharmaceutically acceptable carrier or diluent.

In the present context the term "immunologically effective amount" is to be understood as an amount which elicits a significant protective *Helicobacter pylori*

response, which will eradicate a *H. pylori* infection in an infected mammal or prevent the infection in a susceptible mammal. Typically an immunologically effective amount will comprise approximately 1 µg to 1000 mg, preferably approximately 10 µg to 100 mg, of *H. pylori* antigen for oral administration, or approximately less than 100 µg for parenteral administration.

The vaccine composition comprises optionally in addition to a pharmaceutically acceptable carrier or diluent one or more other immunologically active antigens for prophylactic or therapeutic use. Physiologically acceptable carriers and diluents are well known to those skilled in the art and include e.g. phosphate buffered saline (PBS), or, in the case of oral vaccines, HCO_3^- based formulations or enterically coated powder formulations.

The vaccine composition can optionally include or be administered together with acid secretion inhibitors, preferably proton pump inhibitors (PPIs), e.g. omeprazole. The vaccine can be formulated in known delivery systems such as liposomes, ISCOMs, cochleates, etc. (see e.g. Rabinovich et al. (1994) Science 265, 1401-1404) or be attached to or incorporated into polymer microspheres of degradable or non-degradable nature. The antigens could be associated with live attenuated bacteria, viruses or phages or with killed vectors of the same kind. The antigens can be chemically or genetically coupled to carrier proteins of inert or adjuvantic types (i.e Cholera B subunit). Consequently, the invention provides in a further aspect a vaccine composition according to above, in addition comprising an adjuvant, such as a cholera toxin. Such pharmaceutically acceptable forms of cholera toxin are known in the art, e.g. from Rappuoli et al. (1995) Int. Arch. Allergy & Immunol. 108(4), 327-333; and Dickinson et al. (1995) Infection and Immunity 63(5), 1617-1623.

A vaccine composition according to the invention can be used for both therapeutic and prophylactic purposes. Consequently, the invention includes a vaccine

composition according as defined above, for use as a therapeutic or a prophylactic vaccine in a mammal, including man, which is infected by *Helicobacter pylori*. In this context the term "prophylactic purpose" means to induce an immune response which will protect against future infection by *Helicobacter pylori*, while the term "therapeutic purpose" means to induce an immune response which can eradicate an existing *Helicobacter pylori* infections.

The vaccine composition according to the invention is preferably administered to any mammalian mucosa exemplified by the buccal, the nasal, the tonsillar, the gastric, the intestinal (small and large intestine), the rectal and the vaginal mucosa. The mucosal vaccines can be given together with for the purpose appropriate adjuvants. The vaccine can also be given orally, or parenterally, by the subcutaneous, intracutaneous or intramuscular route, optionally together with the appropriate adjuvant. The vaccine composition can optionally be given together with antimicrobial therapeutic agents.

In a further aspect, the invention proiyides the use of a *Helicobacter pylori* FlgE polypeptide, as defined above, in the manufacture of

(i) a composition for the treatment, prophylaxis or diagnosis of *Helicobacter pylori* infection;

(ii) a vaccine for use in eliciting a protective immune response against *Helicobacter pylori*; and

(iii) a diagnostic kit for diagnosis of *Helicobacter pylori* infection.

In yet a further aspect, the invention provides a method of *in vitro* diagnosis of *Helicobacter pylori* infection comprising at least one step wherein a *Helicobacter pylori* FlgE polypeptide as defined above, optionally labelled or coupled to a solid support, is used. The said method could e.g. comprise the steps (a) contacting a said *Helicobacter pylori* FlgE polypeptide, optionally bound to a solid support, with

a body fluid taken from a mammal; and (b) detecting antibodies from the said body fluid binding to the said FlgE polypeptide. Preferred methods of detecting antibodies are ELISA (Enzyme linked immunoabsorbent assay) methods which are well known in the art.

In another aspect the invention provides a diagnostic kit for the detection of *Helicobacter pylori* infection in a mammal, including man, comprising components which enable the method of *in vitro* diagnosis as described above to be carried out. The said diagnostic kit could e.g. comprise: (a) a *Helicobacter pylori* FlgE polypeptide; and (b) reagents for detecting antibodies binding to the said FlgE polypeptide. The said reagents for detecting antibodies could e.g. be an enzyme-labelled anti-immunoglobulin and a chromogenic substrate for the said enzyme.

In yet a further aspect, the invention provides a method of eliciting in a mammal, including humans, a protective immune response against *Helicobacter pylori* infection, said method comprising the step of administering to the said mammal an immunologically effective amount of a *Helicobacter pylori* FlgE polypeptide as defined above, or alternatively administering to the said mammal an immunologically effective amount of a vaccine composition as defined above.

EXPERIMENTAL METHODS

Throughout this description the terms "standard protocols" and "standard procedures", when used in the context of molecular cloning techniques, are to be understood as protocols and procedures found in an ordinary laboratory manual such as: Current Protocols in Molecular Biology, editors F. Ausubel et al., John Wiley and Sons, Inc. 1994, or Sambrook, J., Fritsch, E.F. and Maniatis, T., Molecular Cloning: A laboratory manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY 1989.

Preparation of recombinant Helicobacter pylori FlgE polypeptide

DNA sequence Information

Sequence information for the gene encoding for the FlgE polypeptide was obtained from the National Center for Biotechnology Information (Accession number U09549; SEQ ID NO: 1).

PCR Amplification and cloning of DNA sequences containing ORF's for membrane and secreted proteins from the J99 Strain of Helicobacter pylori.

Sequences were cloned from the J99 strain of *H. pylori* by amplification cloning using the polymerase chain reaction (PCR). Synthetic oligonucleotide primers (see below) specific for the 5'- and 3'-ends of open reading frames of genes were designed and purchased (GibcoBRL Life Technologies, Gaithersburg, MD, USA). Forward primers (specific for the 5'-end of the sequence) for FlgE were designed to include an *Nco*I cloning site at the extreme 5'-terminus, while reverse primers included a *Eco*RI site at the extreme 5'-terminus to permit cloning of each *H. pylori* sequence into the reading frame of the pET28b vector. Inserts cloned into the *Nco*I-*Eco*RI sites of the pET-28b vector are fused to a vector DNA sequence encoding an additional 20 carboxy-terminal amino including six histidine residues (at the extreme C-terminus).

Forward primer (SEQ ID NO: 3):
5'-TAT ACC ATG GTG CTT AGG TCT TTA T-3'
Reverse primer (SEQ ID NO: 4):
5'-GCG AAT TCA ATT GCT TAA GAT TCA A-3'

Genomic DNA prepared from the J99 strain of *Helicobacter pylori* was used as the source of template DNA for PCR amplification reactions (Current Protocols in Molecular Biology, editors F. Ausubel et al., John Wiley and Sons, Inc. 1994). To amplify a DNA sequence containing an *H. pylori* ORF, genomic DNA (50 ng) was introduced into a reaction vial containing 2 mM $MgCl_2$, 1 µM synthetic oligonucleotide primers (forward and reverse primers) complementary to and flanking a defined *H. pylori* ORF, 0.2 mM of each deoxynucleotide triphosphate dATP, dGTP, dCTP, dTTP, and 2.5 units of heat stable DNA polymerase (Amplitaq, Roche Molecular Systems, Inc., Branchburg, NJ, USA) in a final volume of 100 µl. The following thermal cycling conditions were used to obtain amplified DNA products for each ORF using a Perkin Elmer Cetus/ GeneAmp PCR System 9600 thermal cycler:

Denaturation at +94°C for 2 min;

2 cycles at +94°C for 15 sec, +30°C for 15 sec and +72°C for 1.5 min;

23 cycles at +94°C for 15 sec, +58°C for 15 sec and +72°C for 1.5 min;

Reactions were concluded at +72°C for 6 minutes.

Upon completion of thermal cycling reactions, each sample of amplified DNA was washed and purified using the Qiaquick Spin PCR purification kit (Qiagen, Gaithersburg, MD, USA). Amplified DNA samples were subjected to digestion with the restriction endonucleases *Nde*I and *Eco*RI according to standard procedures. DNA samples were then subjected to electrophoresis on 1.0 % NuSeive (FMC BioProducts, Rockland, ME USA) agarose gels. DNA was visualized by exposure to ethidium bromide and long wave UV irradiation. DNA contained in slices isolated from the agarose gel was purified using the Bio 101 GeneClean Kit protocol (Bio 101 Vista, CA, USA).

Cloning of H. pylori DNA sequences into the pET-28b prokaryotic expression vector.

The pET-28b vector was prepared for cloning by digestion with *Nco*I and *Eco*RI according to standard procedures. Following digestion, DNA inserts were cloned according to standard procedures into the previously digested pET-28b expression vector. Products of the ligation reaction were then used to transform the BL21 strain of *E. coli* as described below.

Transformation of competent bacteria with recombinant plasmids

Competent bacteria, *E. coli* strain BL21 or *E. coli* strain BL21(DE3), were transformed with recombinant pET expression plasmids carrying the cloned *H. pylori* sequences according to standard methods. Briefly, 1 µl of ligation reaction was mixed with 50 µl of electrocompetent cells and subjected to a high voltage pulse, after which, samples were incubated in 0.45 ml SOC medium (0.5% yeast extract, 2.0% tryptone, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$ and 20, mM glucose) at +37°C with shaking for 1 hour. Samples were then spread on LB agar plates containing 25 µg/ml kanamycin sulfate for growth overnight. Transformed colonies of BL21 were then picked and analyzed to evaluate cloned inserts as described below.

Identification of recombinant pET expression plasmids carrying H. pylori sequences

Individual BL21 clones transformed with recombinant pET-28b *H. pylori* genes were analyzed by PCR amplification of the cloned inserts using the same forward and reverse primers, specific for each *H. pylori* sequence, that were used in the original PCR amplification cloning reactions. Successful amplification verified the integration of the *H. pylori* sequences in the expression vector according to standard procedures.

Isolation and Preparation of plasmid DNA from BL21 transformants

Individual clones of recombinant pET-28b vectors carrying properly cloned *H. pylori* ORFs were picked and incubated in 5 ml of LB broth plus 25 μg/ml kanamycin sulfate overnight. The following day plasmid DNA was isolated and purified using the Qiagen plasmid purification protocol (Qiagen Inc., Chatsworth, CA, USA).

Expression of recombinant H. pylori sequences in E. coli

The pET vector can be propagated in any *E. coli* K-12 strain e.g. HMS174, HB101, JM109, DH5α, etc. for the purpose of cloning or plasmid preparation. Hosts for expression include *E. coli* strains containing a chromosomal copy of the gene for T7 RNA polymerase. These hosts are lysogens of bacteriophage DE3, a lambda derivative that carries the *lacI* gene, the lacUV5 promoter and the gene for T7 RNA polymerase. T7 RNA polymerase is induced by addition of isopropyl-β-D-thiogalactoside (IPTG), and the T7 RNA polymerase transcribes any target plasmid, such as pET-28b, carrying its gene of interest. Strains used in our laboratory include: BL21(DE3) (Studier, F.W., Rosenberg, A.H., Dunn, J.J., and Dubendorff, J.W. (1990) Methods Enzymol. 185, 60-89).

To express recombinant *H. pylori* sequences, 50 ng of plasmid DNA isolated as described above was used to transform competent BL21(DE3) bacteria as described above (provided by Novagen as part of the pET expression system kit). Transformed cells were cultured in SOC medium for 1 hour, and the culture was then plated on LB plates containing 25 μg/ml kanamycin sulfate. The following day, bacterial colonies were pooled and grown in LB medium containing kanamycin sulfate (25 μg/ml) to an optical density at 600 nm of 0.5 to 1.0 O.D.

units, at which point, 1 mM IPTG was added to the culture for 3 hours to induce gene expression of the *H. pylori* recombinant DNA constructions .

After induction of gene expression with IPTG, bacteria were pelleted by centrifugation in a Sorvall RC-3B centrifuge at 3500 x g for 15 minutes at 4°C. Pellets were resuspended in 50 ml cold 10 mM Tris-HCl, pH 8.0, 0.1 M NaCl and 0.1 mM EDTA (STE buffer). Cells were then centrifuged at 2000 x g for 20 min at +4°C. Wet pellets were weighed and frozen at –80°C until ready for protein purification.

Analytical Methods

The concentrations of purified protein preparations were quantified spectrophotometrically using absorbance coefficients calculated from amino acid content (Perkins, S.J. 1986 Eur. J. Biochem. 157, 169-180). Protein concentrations were also measured by the method of Bradford, M.M. (1976) Anal.. Biochem. 72, 248-254, and Lowry, O.H., Rosebrough,N., Farr, A.L. & Randall, R.J. (1951) , using bovine serum albumin as a standard.

Sodium dodecyl sulfate-polyacrylamide (SDS-PAGE) gels (12% or 4 to 25 % gradient acrylamide) were purchased from BioRad (Hercules, CA, USA), and stained with Coomassie Brilliant Blue. Molecular mass markers included rabbit skeletal muscle myosin (200 kDa), *E. coli* β-galactosidase (116 kDa), rabbit muscle phosphorylase B (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45 kDa), bovine carbonic anhydrase (31 kDa), soybean trypsin inhibitor (21.5 kDa), egg white lysozyme (14.4 kDa) and bovine aprotinin (6.5 kDa).

Purification of FlgE from inclusion bodies

The following steps were carried out at +4°C. Cell pellets were resuspended in lysis buffer with 10% glycerol 200 µg/ml lysozyme, 5 mM EDTA, 1 mM PMSF and 0.1% β-mercaptoethanol. After passage through the cell disrupter, the resulting homogenate was made 0.2% DOC, stirred 10 minutes, then centrifuged (10,000 g x 30 min). The pellets were first washed with lysis buffer containing 10% glycerol, 10 mM EDTA, 1% Triton X-100, 1 mM PMSF and 0.1% β-mercaptoethanol, then with lysis buffer containing 1 M urea, 1 mM PMSF and 0.1% β-mercaptoethanol. The resulting white pellet was composed primarily of inclusion bodies, free of unbroken cells and membranous materials.

The following steps were carried out at room temperature. Inclusion bodies were dissolved in 20 ml 8 M urea in lysis buffer with 1 mM PMSF and 0.1% β-mercaptoethanol, and incubated at room temperature for 1 hour. Materials that did not dissolve were removed by centrifugation (100,000 x g for 30 min) . The clear supernatant was filtered and loaded onto a Ni^{2+}-NTA agarose column equilibrated in 8 M urea in lysis buffer. The column was washed with 250 ml (50 bed volumes) of lysis buffer containing 8 M urea, 1 mM PMSF and 0.1% β-mercaptoethanol, and developed with sequential steps of lysis buffer containing 8 M urea, 1 mM PMSF, 0.1% β-mercaptoethanol and 20, 100, 200, and 500 mM imidazole. Fractions were monitored by absorbance at OD_{280} nm, and peak fractions were analyzed by SDS-PAGE. Two bands were visualized by Coomassie Brilliant Blue staining, a major band M_r = 78 kDa and a minor band M_r = 60 kDa. Purity of recombinant FlgE (78 kDa) was assessed at greater than 90%. As with the purification of the soluble proteins, fractions containing the recombinant protein eluted at 100 mM imidazole.

Urea was slowly removed from the FlgE polypeptide by dialysis against TBS containing 0.5% DOC with sequential reduction in urea as follows; 6M, 4M, 3M,

2M, 1M, 0.5 M then 0 M. Each dialysis step was carried for a minimum of 4 hours at room temperature,

After dialysis, samples were concentrated by pressure filtration using Amicon stirred cells. Protein concentrations were then measured by the methods of Perkins, Bradford and Lowry.

EXAMPLES OF THE INVENTION

EXAMPLE 1: THERAPEUTIC IMMUNIZATION

1. Materials & Methods

1.1 Animals

Female SPF BALB/c mice were purchased from Bomholt Breeding centre (Denmark). They were kept in ordinary makrolon cages with free supply of water and food. The animals were 4-6 weeks old at arrival.

1.2. Infection

After a minimum of one week of acclimatization, the animals were infected with a type 2 strain of *H. pylori* (strain 244, originally isolated from an ulcer patient). This strain has earlier proven to be a good colonizer of the mouse stomach. Bacteria from a stock kept at –70°C were grown overnight in Brucella broth supplemented with 10% fetal calf serum, at +37°C in a microaerophilic atmosphere (10% CO_2, 5% O_2). The animals were given an oral dose of omeprazole (400 µmol/kg) and after 3-5 h an oral inoculation of *H. pylori* (approximately 10^7-10^8 CFU/animal). Infection was checked in control animals 2-3 weeks after the inoculation.

1.3. Immunizations

One month after infection, two groups of mice (10 mice/group) were immunized 4 times over a 34 day period (day 1, 15, 25 and 35). Purified recombinant FlgE dissolved in PBS plus 0.5% Deoxycholate (DOC) was given at a dose of 100 microgram/mouse.

As an adjuvant, the animals in both the control as well as the FlgE group were also given 10 µg/mouse of cholera toxin (CT) with each immunization. Omeprazole (400 µmol/kg) was given orally to all animals 3-5 h prior to immunization as a way of protecting the antigens from acid degradation. Animals were sacrificed 1-2 weeks after final immunization.

Group 1: 300 µl PBS with 0.5% DOC containing 10 µg CT

Group 2: 300 µl PBS with 0.5% DOC containing 100 µg FlgE and 10 µg CT.

1.4. Analysis of infection

The mice were sacrificed by CO_2 and cervical dislocation. The abdomen and chest cavity was opened and blood sampled by heart puncture. Subsequently the stomach was removed. After cutting the stomach along the greater curvature, it was rinsed in saline and subsequently cut into two identical pieces. An area of 25 mm^2 of the mucosa from the antrum and corpus was scraped separately with a surgical scalpel. The mucosa scraping was suspended in Brucella broth, diluted and plated onto Blood Skirrow plates. The plates were incubated under microaerophilic conditions for 3-5 days and the number of colonies was counted. The identity of *H. pylori* was ascertained by urease and catalase test and by direct microscopy or Gram staining.

1.5. Antibody measurements

Serum antibodies were collected from blood. Prior to centrifugation, the blood was diluted with equal amount of PBS. The serum was kept at –20°C until analysis. Serum antibodies were measured using an ELISA where plates were coated either with a particulate fraction of *H. pylori* strain 244 or with FlgE followed by addition of different dilutions of serum. The ELISA was developed with alkaline phosphatase-labelled anti-mouse-Ig-antibodies. The anti-Ig antibodies were of an anti-heavy/anti-light chain type, which should detect all types of antibodies.

2. Results

2.1. Therapeutic immunization: effects on CFU

The animals in this study were infected with *H. pylori* strain 244 one month prior to immunizations. Mice in groups of ten were then immunized with either cholera toxin (CT) or CT together with the recombinant FlgE polypeptide. Four weeks after the final immunization, the animals were sacrificed and CFU was determined (Fig. 1). The animals treated with CT alone, were highly infected both in corpus and antrum. Animals actively immunized with recombinant FlgE polypeptide and CT had significantly decreased CFU values in the antrum and in the stomach as a whole compared with the CT treated animals ($p<0.01$ and $p<0.05$, respectively; Wilcoxon-Mann-Whittney sign rank test).

2.2. Therapeutic immunization: effects on antibody formation and secretion

As a sign of infection *H. pylori* specific antibodies can be found in serum (Control/244). In animals given FlgE + CT the titer against strain 244 (as membrane proteins) increased 4-fold ($p<0.01$). Only in animals given FlgE + CT could a specific serum IgG titer against FlgE be measured (Fig. 2).

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Astra AB
(B) STREET: Västra Mälarehamnen 9
(C) CITY: Södertälje
(E) COUNTRY: Sweden
(F) POSTAL CODE (ZIP): S-151 85
(G) TELEPHONE: +46 8 553 260 00
(H) TELEFAX: +46 8 553 288 20

(ii) TITLE OF INVENTION: Vaccine Compositions V

(iii) NUMBER OF SEQUENCES: 4

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2550 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Helicobacter pylori

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:321..2477
(D) OTHER INFORMATION:/product= "FlgE flagellar hook protein"

(x) PUBLICATION INFORMATION:
(A) AUTHORS: O'Toole, Paul W.
Kostrzynska, Magdalena
Trust, Trevor J.
(B) TITLE: Non-motile mutants of Helicobacter pylori and Helicobacter mustelae defective in flagellar hook production
(C) JOURNAL: Mol. Microbiol.
(D) VOLUME: 14
(E) ISSUE: 4
(F) PAGES: 691-703
(G) DATE: 1994

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

AACAAAGCGA TAACTCCTTT GTCTTATTAG CGACACAATT TAACCCATTG ACTTTAAATC 60

GCGCTTCAGC CGAAGAGATT CAAGATCATG AATGCGCGAT TTTGCACTAA AGCGAGTTAG 120

ATTCTTAAAT TTGAGCGATA ACCTTTAAAA AGCGTAATTA AGGGGTGGTG TTACAAAACC 180

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CCCTATCCCC TTATGAATTT GACCGATCTT TTTGATTAAC AAAACTTTAA AATCCGCAAT      240

CAATCATTCT AAAAAGCTAT TTAGGAACAA CTTTTGCTTT ATTTTGCATA GATTGAATTT      300

CTTTAAATTA AAGGATAACC ATG CTT AGG TCT TTA TGG TCT GGT GTC AAT           350
                      Met Leu Arg Ser Leu Trp Ser Gly Val Asn
                        1               5                  10

GGG ATG CAA GCC CAC CAA ATC GCT TTG GAT ATT GAG AGT AAC AAT ATT         398
Gly Met Gln Ala His Gln Ile Ala Leu Asp Ile Glu Ser Asn Asn Ile
                   15                  20                  25

GCG AAC GTG AAT ACC ACT GGT TTT AAG TAT TCT AGG GCT TCT TTT GTG         446
Ala Asn Val Asn Thr Thr Gly Phe Lys Tyr Ser Arg Ala Ser Phe Val
               30                  35                  40

GAT ATG CTT TCT CAA GTC AAA CTC ATC GCT ACC GCA CCC TAT AAA AAC         494
Asp Met Leu Ser Gln Val Lys Leu Ile Ala Thr Ala Pro Tyr Lys Asn
           45                  50                  55

GGG TTA GCA GGG CAG AAT GAT TTT TCT GTG GGG CTT GGG GTA GGC GTG         542
Gly Leu Ala Gly Gln Asn Asp Phe Ser Val Gly Leu Gly Val Gly Val
      60                  65                  70

GAT GCG ACG ACT AAA ATC TTT TCA CAA GGC AAT ATC CAA AAC ACA GAT         590
Asp Ala Thr Thr Lys Ile Phe Ser Gln Gly Asn Ile Gln Asn Thr Asp
 75                  80                  85                  90

GTC AAA ACC GAT CTA GCG ATT CAA GGC GAT GGC TTT TTT ATC ATT AAC         638
Val Lys Thr Asp Leu Ala Ile Gln Gly Asp Gly Phe Phe Ile Ile Asn
                95                 100                 105

CCT GAT AGG GGG ATC ACG CGC AAT TTC ACT AGA GAT GGG GAG TTC CTT         686
Pro Asp Arg Gly Ile Thr Arg Asn Phe Thr Arg Asp Gly Glu Phe Leu
           110                 115                 120

TTT GAC TCG CAA GGG AGT TTG GTT ACC ACC GGC GGG CTT GTG GTG CAA         734
Phe Asp Ser Gln Gly Ser Leu Val Thr Thr Gly Gly Leu Val Val Gln
      125                 130                 135

GGG TGG GTG AGA AAT GGG AGC GAT ACC GGC AAT AAA GGG AGC GAT ACA         782
Gly Trp Val Arg Asn Gly Ser Asp Thr Gly Asn Lys Gly Ser Asp Thr
  140                 145                 150

GAC GCT TTA AAA GTG GAT AAC ACC GGT CCT TTA GAA AAC ATT AGG ATT         830
Asp Ala Leu Lys Val Asp Asn Thr Gly Pro Leu Glu Asn Ile Arg Ile
155                 160                 165                 170

GAT CCT GGA ATG GTG ATG CCA GCC AGA GCG AGT AAC CGC ATT TCT ATG         878
Asp Pro Gly Met Val Met Pro Ala Arg Ala Ser Asn Arg Ile Ser Met
                175                 180                 185

AGG GCG AAT TTA AAC GCT GGA AGG CAT GCC GAT CAA ACA GCG GCG ATA         926
Arg Ala Asn Leu Asn Ala Gly Arg His Ala Asp Gln Thr Ala Ala Ile
           190                 195                 200

TTC GCT TTG GAT TCT TCA GCC AAA ACC CCT TCA GAT GGC ATT AAT CCG         974
Phe Ala Leu Asp Ser Ser Ala Lys Thr Pro Ser Asp Gly Ile Asn Pro
      205                 210                 215

GTG TAT GAT TCA GGC ACG AAT CTT GCT CAA GTC GCC GAA GAC ATG GGA        1022
Val Tyr Asp Ser Gly Thr Asn Leu Ala Gln Val Ala Glu Asp Met Gly
  220                 225                 230

TCT TTA TAC AAT GAA GAT GGC GAC GCT CTT TTG TTG AAT GAA AAT CAA        1070
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Ser Leu Tyr Asn Glu Asp Gly Asp Ala Leu Leu Leu Asn Glu Asn Gln
235                 240                 245                 250

GGG ATT TGG GTG AGC TAT AAG AGT CCA AAA ATG GTC AAA GAC ATC CTC      1118
Gly Ile Trp Val Ser Tyr Lys Ser Pro Lys Met Val Lys Asp Ile Leu
                255                 260                 265

CCT TCT GCA GAA AAC AGC ACG CTT GAA TTG AAT GGC GTT AAG ATT TCT      1166
Pro Ser Ala Glu Asn Ser Thr Leu Glu Leu Asn Gly Val Lys Ile Ser
            270                 275                 280

TTC ACA AAC GAT TCA GCG GTG AGC CGG ACT TCA AGC TTA GTG GCG GCT      1214
Phe Thr Asn Asp Ser Ala Val Ser Arg Thr Ser Ser Leu Val Ala Ala
        285                 290                 295

AAA AAT GCG ATC AAT GCA GTC AAA AGC CAA ACA GGC ATT GAA GCT TAT      1262
Lys Asn Ala Ile Asn Ala Val Lys Ser Gln Thr Gly Ile Glu Ala Tyr
    300                 305                 310

TTA GAC GGC AAG CAA TTG CGT TTG GAA AAC ACC AAT GAA TTA GAC GGC      1310
Leu Asp Gly Lys Gln Leu Arg Leu Glu Asn Thr Asn Glu Leu Asp Gly
315                 320                 325                 330

GAT GAA AAG CTT AAA AAC ATT GTA GTT ACT CAA GCC GGA ACC GGA GCG      1358
Asp Glu Lys Leu Lys Asn Ile Val Val Thr Gln Ala Gly Thr Gly Ala
                335                 340                 345

TTC GCT AAC TTT TTA GAC GGC GAT AAA GAT GTA ACG GCT TTC AAA TAC      1406
Phe Ala Asn Phe Leu Asp Gly Asp Lys Asp Val Thr Ala Phe Lys Tyr
            350                 355                 360

AGC TAC ACG CAT TCT ATT AGC CCT AAC GCC AAT AGC GGG CAG TTT AGG      1454
Ser Tyr Thr His Ser Ile Ser Pro Asn Ala Asn Ser Gly Gln Phe Arg
        365                 370                 375

ACC ACT GAA GAC TTG CGC GCC TTA ATC CAG CAT GAC GCT AAT ATC GTT      1502
Thr Thr Glu Asp Leu Arg Ala Leu Ile Gln His Asp Ala Asn Ile Val
    380                 385                 390

AAA GAT CCT AGC CTA GCG GAC AAT TAC CAA GAC TCA GCC GCT TCT ATA      1550
Lys Asp Pro Ser Leu Ala Asp Asn Tyr Gln Asp Ser Ala Ala Ser Ile
395                 400                 405                 410

GGA GTT ACA ATC AAC CAA TAC GGC ATG TTT GAA ATC AAC AAT AAA GAC      1598
Gly Val Thr Ile Asn Gln Tyr Gly Met Phe Glu Ile Asn Asn Lys Asp
                415                 420                 425

AAT AAA AAT GTC ATT AAA GAA AAT CTT AAT ATC TTT GTG AGC GGG TAT      1646
Asn Lys Asn Val Ile Lys Glu Asn Leu Asn Ile Phe Val Ser Gly Tyr
            430                 435                 440

TCT TCA GAC AGC GTA ACG AAC AAT GTT TTG TTT AAA AAT GCG ATG AAA      1694
Ser Ser Asp Ser Val Thr Asn Asn Val Leu Phe Lys Asn Ala Met Lys
        445                 450                 455

GGG CTT AAT ACC GCT TCT TTA ATT GAA GGG GGA GCG TCA GCG AGC AGT      1742
Gly Leu Asn Thr Ala Ser Leu Ile Glu Gly Gly Ala Ser Ala Ser Ser
    460                 465                 470

TCT AAA TTC ACC CAC GCT ACG CAT GCG ACA AGC ATT GAT GTG ATA GAC      1790
Ser Lys Phe Thr His Ala Thr His Ala Thr Ser Ile Asp Val Ile Asp
475                 480                 485                 490

AGC TTA GGC ACT AAA CAC GCC ATG CGC ATT GAG TTT TAT AGG AGT GGG      1838
Ser Leu Gly Thr Lys His Ala Met Arg Ile Glu Phe Tyr Arg Ser Gly
                495                 500                 505
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GGA GCG GAT TGG AAT TTT AGA GTG ATC GTG CCT GAG CCT GGG GAA TTA      1886
Gly Ala Asp Trp Asn Phe Arg Val Ile Val Pro Glu Pro Gly Glu Leu
            510                 515                 520

GTA GGG GGG TCA GCG GCT AGG CCT AAT GTG TTT GAA GGA GGC CGT TTG      1934
Val Gly Gly Ser Ala Ala Arg Pro Asn Val Phe Glu Gly Gly Arg Leu
        525                 530                 535

CAC TTC AAT AAT GAC GGA TCG CTT GCA GGC ATG AAC CCG CCT CTT TTG      1982
His Phe Asn Asn Asp Gly Ser Leu Ala Gly Met Asn Pro Pro Leu Leu
    540                 545                 550

CAA TTT GAC CCT AAA AAT GGT GCT GAT GCC CCC CAA CGC ATC AAT TTA      2030
Gln Phe Asp Pro Lys Asn Gly Ala Asp Ala Pro Gln Arg Ile Asn Leu
555                 560                 565                 570

GCT TTT GGT TCC TCA GGG AGT TTT GAC GGG CTA ACG AGC GTG GAT AAG      2078
Ala Phe Gly Ser Ser Gly Ser Phe Asp Gly Leu Thr Ser Val Asp Lys
                575                 580                 585

ATT TCT GAA ACT TAT GCG ATT GAG CAA AAC GGC TAT CAA GCG GGC GAT      2126
Ile Ser Glu Thr Tyr Ala Ile Glu Gln Asn Gly Tyr Gln Ala Gly Asp
            590                 595                 600

TTG ATG GAT GTC CGC TTT GAT TCA GAT GGG GTG CTT TTA GGA GCG TTC      2174
Leu Met Asp Val Arg Phe Asp Ser Asp Gly Val Leu Leu Gly Ala Phe
        605                 610                 615

AGT AAT GGC AGG ACT TTA GCG CTC GCT CAA GTG GCT TTA GCG AAT TTC      2222
Ser Asn Gly Arg Thr Leu Ala Leu Ala Gln Val Ala Leu Ala Asn Phe
    620                 625                 630

GCT AAC GAT GCG GGC TTG CAG GCT TTA GGC GGG AAT GTC TTT TCT CAA      2270
Ala Asn Asp Ala Gly Leu Gln Ala Leu Gly Gly Asn Val Phe Ser Gln
635                 640                 645                 650

ACC GGA AAC TCA GGG CAA GCC TTA ATC GGT GCG GCT AAT ACG GGG CGT      2318
Thr Gly Asn Ser Gly Gln Ala Leu Ile Gly Ala Ala Asn Thr Gly Arg
                655                 660                 665

AGG GGT TCA ATT TCA GGA TCT AAA CTG GAG TCT AGT AAT GTG GAT TTG      2366
Arg Gly Ser Ile Ser Gly Ser Lys Leu Glu Ser Ser Asn Val Asp Leu
            670                 675                 680

AGC CGG AGT TTA ACG AAT TTG ATT GTG GTT CAA AGG GGC TTT CAA GCA      2414
Ser Arg Ser Leu Thr Asn Leu Ile Val Val Gln Arg Gly Phe Gln Ala
        685                 690                 695

AAC TCT AAA GCG GTA ACC ACA TCC GAT CAA ATC CTT AAT ACC CTA TTG      2462
Asn Ser Lys Ala Val Thr Thr Ser Asp Gln Ile Leu Asn Thr Leu Leu
    700                 705                 710

AAT CTT AAG CAA TAA ACTAAAGGAT TACTCTAATA CAATATAATA GGGGCTAATT      2517
Asn Leu Lys Gln  *
715

TAAAGATTAA GGTTTAGTAT GCATGAATAC TCG                                2550
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 719 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

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GGA GCG GAT TGG AAT TTT AGA GTG ATC GTG CCT GAG CCT GGG GAA TTA      1886
Gly Ala Asp Trp Asn Phe Arg Val Ile Val Pro Glu Pro Gly Glu Leu
            510                 515                 520

GTA GGG GGG TCA GCG GCT AGG CCT AAT GTG TTT GAA GGA GGC CGT TTG      1934
Val Gly Gly Ser Ala Ala Arg Pro Asn Val Phe Glu Gly Gly Arg Leu
        525                 530                 535

CAC TTC AAT AAT GAC GGA TCG CTT GCA GGC ATG AAC CCG CCT CTT TTG      1982
His Phe Asn Asn Asp Gly Ser Leu Ala Gly Met Asn Pro Pro Leu Leu
    540                 545                 550

CAA TTT GAC CCT AAA AAT GGT GCT GAT GCC CCC CAA CGC ATC AAT TTA      2030
Gln Phe Asp Pro Lys Asn Gly Ala Asp Ala Pro Gln Arg Ile Asn Leu
555                 560                 565                 570

GCT TTT GGT TCC TCA GGG AGT TTT GAC GGG CTA ACG AGC GTG GAT AAG      2078
Ala Phe Gly Ser Ser Gly Ser Phe Asp Gly Leu Thr Ser Val Asp Lys
                575                 580                 585

ATT TCT GAA ACT TAT GCG ATT GAG CAA AAC GGC TAT CAA GCG GGC GAT      2126
Ile Ser Glu Thr Tyr Ala Ile Glu Gln Asn Gly Tyr Gln Ala Gly Asp
            590                 595                 600

TTG ATG GAT GTC CGC TTT GAT TCA GAT GGG GTG CTT TTA GGA GCG TTC      2174
Leu Met Asp Val Arg Phe Asp Ser Asp Gly Val Leu Leu Gly Ala Phe
        605                 610                 615

AGT AAT GGC AGG ACT TTA GCG CTC GCT CAA GTG GCT TTA GCG AAT TTC      2222
Ser Asn Gly Arg Thr Leu Ala Leu Ala Gln Val Ala Leu Ala Asn Phe
    620                 625                 630

GCT AAC GAT GCG GGC TTG CAG GCT TTA GGC GGG AAT GTC TTT TCT CAA      2270
Ala Asn Asp Ala Gly Leu Gln Ala Leu Gly Gly Asn Val Phe Ser Gln
635                 640                 645                 650

ACC GGA AAC TCA GGG CAA GCC TTA ATC GGT GCG GCT AAT ACG GGG CGT      2318
Thr Gly Asn Ser Gly Gln Ala Leu Ile Gly Ala Ala Asn Thr Gly Arg
                655                 660                 665

AGG GGT TCA ATT TCA GGA TCT AAA CTG GAG TCT AGT AAT GTG GAT TTG      2366
Arg Gly Ser Ile Ser Gly Ser Lys Leu Glu Ser Ser Asn Val Asp Leu
            670                 675                 680

AGC CGG AGT TTA ACG AAT TTG ATT GTG GTT CAA AGG GGC TTT CAA GCA      2414
Ser Arg Ser Leu Thr Asn Leu Ile Val Val Gln Arg Gly Phe Gln Ala
        685                 690                 695

AAC TCT AAA GCG GTA ACC ACA TCC GAT CAA ATC CTT AAT ACC CTA TTG      2462
Asn Ser Lys Ala Val Thr Thr Ser Asp Gln Ile Leu Asn Thr Leu Leu
    700                 705                 710

AAT CTT AAG CAA TAA ACTAAAGGAT TACTCTAATA CAATATAATA GGGGCTAATT      2517
Asn Leu Lys Gln  *
715

TAAAGATTAA GGTTTAGTAT GCATGAATAC TCG                                2550
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 719 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Leu Arg Ser Leu Trp Ser Gly Val Asn Gly Met Gln Ala His Gln
1 5 10 15

Ile Ala Leu Asp Ile Glu Ser Asn Asn Ile Ala Asn Val Asn Thr Thr
20 25 30

Gly Phe Lys Tyr Ser Arg Ala Ser Phe Val Asp Met Leu Ser Gln Val
35 40 45

Lys Leu Ile Ala Thr Ala Pro Tyr Lys Asn Gly Leu Ala Gly Gln Asn
50 55 60

Asp Phe Ser Val Gly Leu Gly Val Gly Val Asp Ala Thr Thr Lys Ile
65 70 75 80

Phe Ser Gln Gly Asn Ile Gln Asn Thr Asp Val Lys Thr Asp Leu Ala
85 90 95

Ile Gln Gly Asp Gly Phe Phe Ile Ile Asn Pro Asp Arg Gly Ile Thr
100 105 110

Arg Asn Phe Thr Arg Asp Gly Glu Phe Leu Phe Asp Ser Gln Gly Ser
115 120 125

Leu Val Thr Thr Gly Gly Leu Val Val Gln Gly Trp Val Arg Asn Gly
130 135 140

Ser Asp Thr Gly Asn Lys Gly Ser Asp Thr Asp Ala Leu Lys Val Asp
145 150 155 160

Asn Thr Gly Pro Leu Glu Asn Ile Arg Ile Asp Pro Gly Met Val Met
165 170 175

Pro Ala Arg Ala Ser Asn Arg Ile Ser Met Arg Ala Asn Leu Asn Ala
180 185 190

Gly Arg His Ala Asp Gln Thr Ala Ala Ile Phe Ala Leu Asp Ser Ser
195 200 205

Ala Lys Thr Pro Ser Asp Gly Ile Asn Pro Val Tyr Asp Ser Gly Thr
210 215 220

Asn Leu Ala Gln Val Ala Glu Asp Met Gly Ser Leu Tyr Asn Glu Asp
225 230 235 240

Gly Asp Ala Leu Leu Leu Asn Glu Asn Gln Gly Ile Trp Val Ser Tyr
245 250 255

Lys Ser Pro Lys Met Val Lys Asp Ile Leu Pro Ser Ala Glu Asn Ser
260 265 270

Thr Leu Glu Leu Asn Gly Val Lys Ile Ser Phe Thr Asn Asp Ser Ala
275 280 285

Val Ser Arg Thr Ser Ser Leu Val Ala Ala Lys Asn Ala Ile Asn Ala
290 295 300

Val Lys Ser Gln Thr Gly Ile Glu Ala Tyr Leu Asp Gly Lys Gln Leu
305 310 315 320

Arg Leu Glu Asn Thr Asn Glu Leu Asp Gly Asp Glu Lys Leu Lys Asn

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Leu Arg Ser Leu Trp Ser Gly Val Asn Gly Met Gln Ala His Gln
1 5 10 15

Ile Ala Leu Asp Ile Glu Ser Asn Asn Ile Ala Asn Val Asn Thr Thr
20 25 30

Gly Phe Lys Tyr Ser Arg Ala Ser Phe Val Asp Met Leu Ser Gln Val
35 40 45

Lys Leu Ile Ala Thr Ala Pro Tyr Lys Asn Gly Leu Ala Gly Gln Asn
50 55 60

Asp Phe Ser Val Gly Leu Gly Val Gly Val Asp Ala Thr Thr Lys Ile
65 70 75 80

Phe Ser Gln Gly Asn Ile Gln Asn Thr Asp Val Lys Thr Asp Leu Ala
85 90 95

Ile Gln Gly Asp Gly Phe Phe Ile Ile Asn Pro Asp Arg Gly Ile Thr
100 105 110

Arg Asn Phe Thr Arg Asp Gly Glu Phe Leu Phe Asp Ser Gln Gly Ser
115 120 125

Leu Val Thr Thr Gly Gly Leu Val Val Gln Gly Trp Val Arg Asn Gly
130 135 140

Ser Asp Thr Gly Asn Lys Gly Ser Asp Thr Asp Ala Leu Lys Val Asp
145 150 155 160

Asn Thr Gly Pro Leu Glu Asn Ile Arg Ile Asp Pro Gly Met Val Met
165 170 175

Pro Ala Arg Ala Ser Asn Arg Ile Ser Met Arg Ala Asn Leu Asn Ala
180 185 190

Gly Arg His Ala Asp Gln Thr Ala Ala Ile Phe Ala Leu Asp Ser Ser
195 200 205

Ala Lys Thr Pro Ser Asp Gly Ile Asn Pro Val Tyr Asp Ser Gly Thr
210 215 220

Asn Leu Ala Gln Val Ala Glu Asp Met Gly Ser Leu Tyr Asn Glu Asp
225 230 235 240

Gly Asp Ala Leu Leu Leu Asn Glu Asn Gln Gly Ile Trp Val Ser Tyr
245 250 255

Lys Ser Pro Lys Met Val Lys Asp Ile Leu Pro Ser Ala Glu Asn Ser
260 265 270

Thr Leu Glu Leu Asn Gly Val Lys Ile Ser Phe Thr Asn Asp Ser Ala
275 280 285

Val Ser Arg Thr Ser Ser Leu Val Ala Ala Lys Asn Ala Ile Asn Ala
290 295 300

Val Lys Ser Gln Thr Gly Ile Glu Ala Tyr Leu Asp Gly Lys Gln Leu
305 310 315 320

Arg Leu Glu Asn Thr Asn Glu Leu Asp Gly Asp Glu Lys Leu Lys Asn

325 330 335

Ile Val Val Thr Gln Ala Gly Thr Gly Ala Phe Ala Asn Phe Leu Asp
340 345 350

Gly Asp Lys Asp Val Thr Ala Phe Lys Tyr Ser Tyr Thr His Ser Ile
355 360 365

Ser Pro Asn Ala Asn Ser Gly Gln Phe Arg Thr Thr Glu Asp Leu Arg
370 375 380

Ala Leu Ile Gln His Asp Ala Asn Ile Val Lys Asp Pro Ser Leu Ala
385 390 395 400

Asp Asn Tyr Gln Asp Ser Ala Ala Ser Ile Gly Val Thr Ile Asn Gln
405 410 415

Tyr Gly Met Phe Glu Ile Asn Asn Lys Asp Asn Lys Asn Val Ile Lys
420 425 430

Glu Asn Leu Asn Ile Phe Val Ser Gly Tyr Ser Ser Asp Ser Val Thr
435 440 445

Asn Asn Val Leu Phe Lys Asn Ala Met Lys Gly Leu Asn Thr Ala Ser
450 455 460

Leu Ile Glu Gly Gly Ala Ser Ala Ser Ser Ser Lys Phe Thr His Ala
465 470 475 480

Thr His Ala Thr Ser Ile Asp Val Ile Asp Ser Leu Gly Thr Lys His
485 490 495

Ala Met Arg Ile Glu Phe Tyr Arg Ser Gly Gly Ala Asp Trp Asn Phe
500 505 510

Arg Val Ile Val Pro Glu Pro Gly Glu Leu Val Gly Gly Ser Ala Ala
515 520 525

Arg Pro Asn Val Phe Glu Gly Gly Arg Leu His Phe Asn Asn Asp Gly
530 535 540

Ser Leu Ala Gly Met Asn Pro Pro Leu Leu Gln Phe Asp Pro Lys Asn
545 550 555 560

Gly Ala Asp Ala Pro Gln Arg Ile Asn Leu Ala Phe Gly Ser Ser Gly
565 570 575

Ser Phe Asp Gly Leu Thr Ser Val Asp Lys Ile Ser Glu Thr Tyr Ala
580 585 590

Ile Glu Gln Asn Gly Tyr Gln Ala Gly Asp Leu Met Asp Val Arg Phe
595 600 605

Asp Ser Asp Gly Val Leu Leu Gly Ala Phe Ser Asn Gly Arg Thr Leu
610 615 620

Ala Leu Ala Gln Val Ala Leu Ala Asn Phe Ala Asn Asp Ala Gly Leu
625 630 635 640

Gln Ala Leu Gly Gly Asn Val Phe Ser Gln Thr Gly Asn Ser Gly Gln
645 650 655

Ala Leu Ile Gly Ala Ala Asn Thr Gly Arg Arg Gly Ser Ile Ser Gly
660 665 670

Ser Lys Leu Glu Ser Ser Asn Val Asp Leu Ser Arg Ser Leu Thr Asn

675 680 685

Leu Ile Val Val Gln Arg Gly Phe Gln Ala Asn Ser Lys Ala Val Thr
690 695 700

Thr Ser Asp Gln Ile Leu Asn Thr Leu Leu Asn Leu Lys Gln *
705 710 715

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "PCR primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

TATACCATGG TGCTTAGGTC TTTAT 25

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "PCR primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GCGAATTCAA TTGCTTAAGA TTCAA 25

CLAIMS

1. A *Helicobacter pylori* FlgE polypeptide, or a modified form thereof retaining functionally equivalent antigenicity, for use in inducing a protective immune response to *Helicobacter pylori* infection.

2. A *Helicobacter pylori* FlgE polypeptide according to claim 1 which has substantially the amino acid sequence shown in SEQ ID NO: 2 in the Sequence Listing, for use in inducing a protective immune response to *Helicobacter pylori* infection.

3. A vaccine composition for inducing a protective immune response to *Helicobacter pylori* infection, comprising an immunogenically effective amount of a *Helicobacter pylori* FlgE polypeptide as defined in claim 1 or 2, optionally together with a pharmaceutically acceptable carrier or diluent.

4. A vaccine composition according to claim 3 in addition comprising an adjuvant.

5. A vaccine composition according to claim 4 wherein the adjuvant is a pharmaceutically acceptable form of cholera toxin.

6. A vaccine composition according to any one of claims 3 to 5 for use as a therapeutic vaccine in a mammal, including man, which is infected by *Helicobacter pylori*.

7. A vaccine composition according to any one of claims 3 to 5 for use as a prophylactic vaccine to protect a mammal, including man, from infection by *Helicobacter pylori*.

8. Use of a *Helicobacter pylori* FlgE polypeptide as defined in claim 1 or 2 in the manufacture of a composition for the treatment, prophylaxis or diagnosis of *Helicobacter pylori* infection.

9. Use of a *Helicobacter pylori* FlgE polypeptide as defined in claim 1 or 2 in the manufacture of a vaccine for use in eliciting a protective immune response against *Helicobacter pylori*.

10. Use of a *Helicobacter pylori* FlgE polypeptide as defined in claim 1 or 2 in the manufacture of a diagnostic kit for diagnosis of *Helicobacter pylori* infection.

11. A method of *in vitro* diagnosis of *Helicobacter pylori* infection comprising at least one step wherein a *Helicobacter pylori* FlgE polypeptide as defined in claim 1 or 2, optionally labelled or coupled to a solid support, is used.

12. A method according to claim 11 comprising the steps

(a) contacting a said *Helicobacter pylori* FlgE polypeptide, optionally bound to a solid support, with a body fluid taken from a mammal; and

(b) detecting antibodies from the said body fluid binding to the said FlgE polypeptide.

13. A diagnostic kit for the detection of *Helicobacter pylori* infection in a mammal, including man, comprising components which enable the method according to claim 11 or 12 to be carried out.

14. A diagnostic kit according to claim 13, comprising:

(a) a *Helicobacter pylori* FlgE polypeptide; and

(b) reagents for detecting antibodies binding to the said FlgE polypeptide.

15. A method of eliciting in a mammal a protective immune response against *Helicobacter pylori* infection, said method comprising the step of administering to the said mammal an immunologically effective amount of a *Helicobacter pylori* FlgE polypeptide as defined in claim 1 or 2.

16. A method of eliciting in a mammal a protective immune response against *Helicobacter pylori* infection, said method comprising the step of administering to the said mammal an immunologically effective amount of a vaccine composition according to any one of claims 3 to 7.

17. A method according to claim 15 or 16 wherein the said mammal is a human.

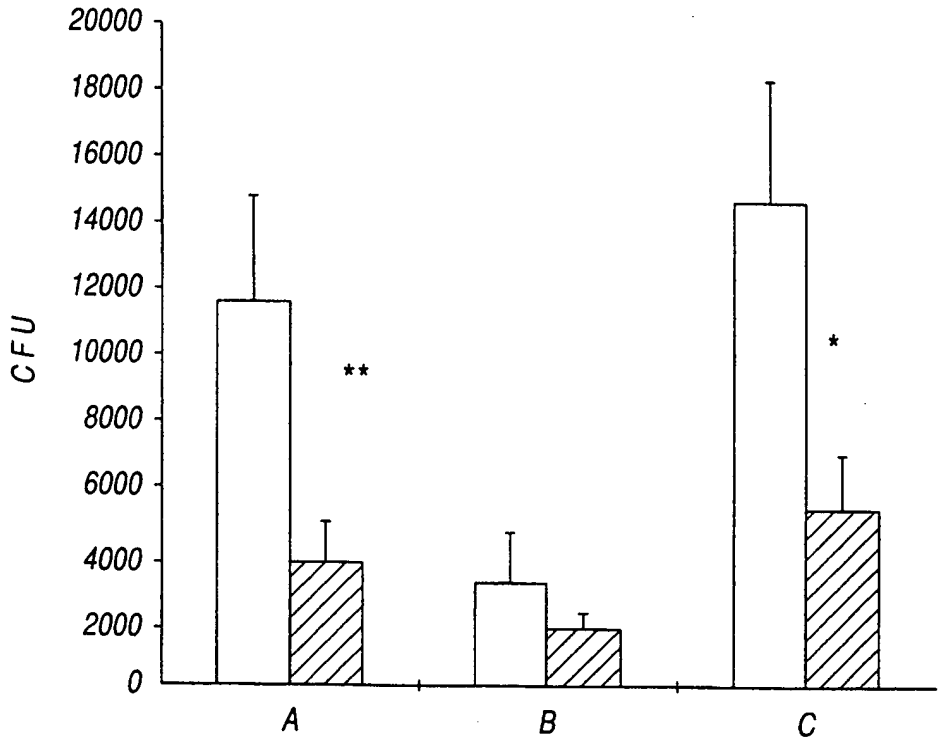
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Fig.1

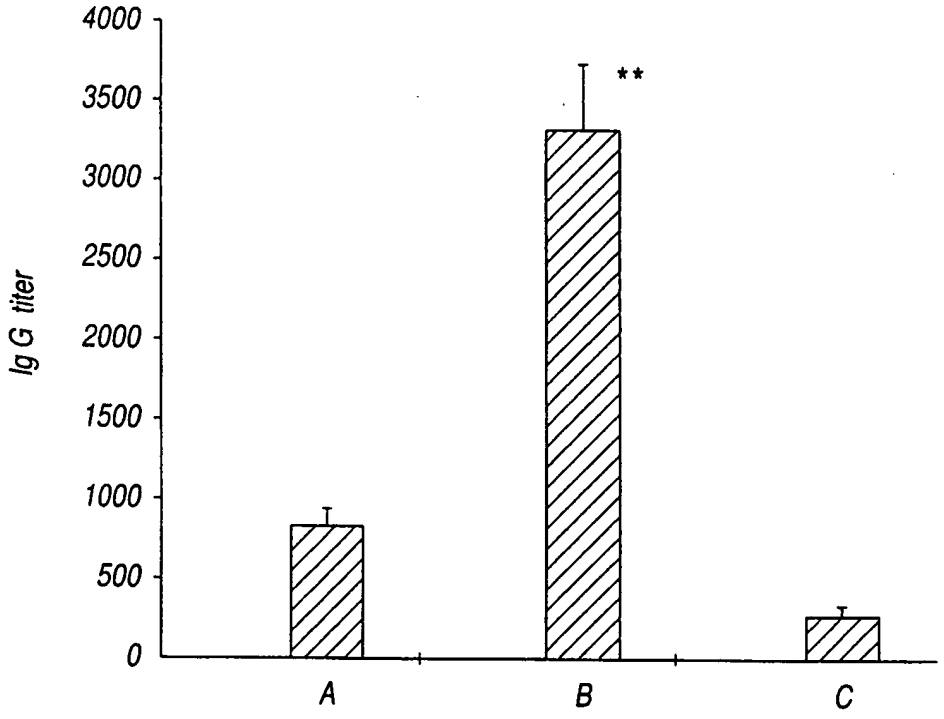

Fig.2